COMPARISON OF PHOTOSYNTHETIC RESPONSES OF ASHE JUNIPER AND LIVE OAK ON THE EDWARDS PLATEAU, TEXAS

A Thesis

by

MIRA ARPE BENDEVIS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2006

Major Subject: Agronomy

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Approved by:

Co-Chairs of Committee,	M. Keith Owens
	James L. Heilman
Committee Member,	J. Tom Cothren
Head of Department,	C.W. Smith

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ABSTRACT

Comparison of Photosynthetic Responses of Ashe Juniper and Live Oak on the Edwards Plateau, Texas. (August 2006)

Mira Arpe Bendevis, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. M. Keith Owens
Dr. James L. Heilman

Ashe juniper (*Juniperus ashei* Bucholz) has encroached into the historical grasslands of the Edwards Plateau. This area is environmentally sensitive as it serves as the recharge zone for the Edwards aquifer, providing large municipalities such as Austin, San Antonio, and San Marcos with water. The increased tree density may impact local water budgets, but the trees may have the capability of sequestering a greater amount of carbon than the historic grasslands. An understanding of what regulates gas exchange and water relations at the leaf level of the two dominant tree species, Ashe juniper and live oak (*Quercus virginiana* P. Mill. Var. *fusiformis*), is important to assess the impact of juniper encroachment on the aquifer. Photosynthesis and transpiration were measured in four juniper and four oak trees throughout an entire year. Juniper consistently had lower carbon assimilation rates, transpiration, and conductance values than oak. Oak exhibited greater seasonal variation and seemed less dependent on precipitation to maintain gas exchange. Canopy position in live oak regulates leaf level photosynthesis to a higher degree than in Ashe juniper. Gas exchange of both species decline as water becomes limited, but juniper consistently exhibits lower and steadier rates throughout the year than oak. Juniper does not respond quickly to erratic precipitation events. The

consistent low rates of gas exchange and stomatal responses in juniper could indicate shallower rooting structure and/or limitation of hydraulic conductivity, as well as photosynthetic capacity. The higher rates of photosynthesis, transpiration, and stomatal conductivity exhibited by live oak during drought suggest a deeper rooting pattern than Ashe juniper. Light response curves were computed for three juniper and three oak trees that were marked and sampled at three different seasons. Juniper light-compensation and light-saturation estimates, at different canopy positions, were poor indicators of differences in photosynthetic capacity between the two species. Patterns of light responses of juniper and oak did not follow responses of previous studies assessing differences in photosynthetic capacity through light-response curves. Computing the light curves was difficult due to low tree activity and lack of responses to changes in light, especially in juniper.

ACKNOWLEDGEMENTS

This thesis could not have been written nor my research done without the help of quite a number of people. First and foremost I would like to thank Dr. Owens for his guidance through this process, numerous hours of editing everything I wrote, helping with data analysis, which made my brain hurt, and going above and beyond by risking his own life during numerous days of data collection. Dr. Heilman probably deserves a medal for hiring me, for all his help with administrative issues, and for being a good sport about my frequent escapes to the East Coast. I must thank Dr. Cothren for serving on my committee and for letting me borrow valuable equipment which without, none of this data would have been collected. Gratitude also goes to Amy Neuenschwander at the University of Texas, for providing the much needed LAI values and to Dr. Don Vietor, for allowing me the use of equipment and access to the lab at odd hours. I must thank fellow indentured servant Lisa Dreiling, for being my faithful helper in the field, as well as Rose Cooper and Bobby Ayala for their efforts. My family and friends are recognized for their support and encouragement, which was needed when frustration caused by mysteriously eaten leaves or bad weather would get the better of me. The LiCor support staff was much appreciated for kindly taking the time to help out lost graduate students without making any remarks about stupid questions. Last but not least, thanks to Dr. Kjelgaard for constantly reminding me of the end goal, becoming a Master of Science.

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CHAPTER I

INTRODUCTION

Encroachment by introduced or native species is common in ecosystems throughout the world (Auken 2000). Particularly noticeable is the increase of woody plants in ecosystems that historically have been dominated by grasses (Norris et al. 2001). The Edwards Plateau of central Texas is a prime example of this phenomenon (Jessup et al. 2003). The Edwards Plateau is a vast (93,000 km^2) biome which serves as the drainage area and recharge zone for the Edwards Aquifer as well as being home to endangered and endemic plant and animal species. The vegetation on the plateau has historically been dominated by live oak (*Quercus virginiana* P. Mill. var. *fusiformis*) - Ashe juniper (*Juniperus ashei* Buchh.) semi-arid savanna and C_4 grassland (Barnes et al. 2000). Due to changes in management, suppression of naturally occurring fires, preferential grazing, and most importantly the invasive nature of Ashe juniper (Adams et al. 1998), Plateau grasslands have been reduced and Ashe juniper populations have increased (Jessup et al. 2003). *Juniperus* in Texas provide examples of invasive species, as they have shown a noticeable increase in their range and density during the last 200 years.

This thesis follows the format and style of *Rangeland Ecology and Management.*

Concurrently *Quercus* has also expanded its habitat throughout the state, but *Juniperus* is becoming dominant in mixed woodland biomes (Smeins and Merrill 1988). This composition of woody species is unique to the Edwards Plateau and Northeastern Mexico.

The Plateau is predominantly limestone with shallow and rocky soil on slopes. The area is subject to periodic drought and extreme flooding and most parts are used for grazing and hunting (Barnes et al. 2000). However, in spite of its ecological and economic significance, the specific structure and function of the existing Edwards Plateau ecosystems are not well understood, nor have the ecological consequences of changes been completely assessed. Magnitude and direction of the changes in carbon balance due to a shift from grassland to woody vegetation are unknown, and no long term studies have been made in the area.

Atmospheric CO_2 concentration continues to increase globally by about 1 ppm per year due to continued anthropogenic emissions (Cohen et al. 2001; Taiz and Zeiger 2002). Due to this carbon dioxide increase, global climate change models predict an increase in average air temperature resulting in a change in precipitation patterns. The shift is predicted to result in an increase in winter/spring precipitation and a decrease in summer precipitation for this region of Texas (Schneider et al. 1993; North 1995; Kattenberg et al. 1996). The predicted change in climate patterns have caused concern for potential impacts as it may be important to the distribution and establishment of vegetation, especially in semi-arid environments where water availability is a limiting growth factor (Hanson and Weltzin 2000; Perkins and Owens 2003).

This study was a part of a long-term study designed to quantify, compare, and identify carbon dioxide and energy fluxes, and processes controlling carbon dioxide exchange in live oak-Ashe juniper woodland and in a grassland ecosystem on the Edwards Plateau. To gain an understanding of the mechanisms adding to the success and competition of the two dominant tree species as well as partitioning their contribution to whole ecosystem energy flux, the objectives of this research were to:

1. Compare relative leaf net photosynthesis of Ashe juniper and live oak and relate that to seasonal and environmental influences that may possibly influence species distribution within an oak-juniper system.
2. Determine the degree to which canopy leaf position plays a role in regulating leaf-level light responses of the two site dominant tree species, Ashe juniper and live oak.
3. Scale leaf level data to the whole-tree level to compare relative contributions of Ashe juniper and live oak trees to ecosystem gas exchange.

CHAPTER II

SEASONAL GAS EXCHANGE

Introduction

Live oak (*Quercus virginiana* var. *fusiformis)* and Ashe juniper (*Juniperus ashei)* are the two woody species dominating the Edwards Plateau of central Texas. Recently live oak populations have been stable or exhibited a slight decline while Ashe juniper appears, in some instances, to be increasing and possibly replacing the oak trees (Russell and Fowler 1999). Invasive species typically have high photosynthetic rates and/or distinct seasonal patterns of growth combined with specialized life history strategies such as rapid reproduction and broad seed dispersal (McDowell 2002; Deng et al. 2004) which allow them to out-compete native species. However, Ashe juniper does not possess any of those characteristics, which makes it unclear how juniper can be encroaching on the Edwards Plateau. Ashe juniper life history strategies should not confer a competitive advantage over live oak (Owens and Schliesing 1995). Because both species are evergreen and photosynthetically active throughout the year, distinct seasonal patterns of gas exchange should not be an important difference. Relative drought tolerance, water use efficiency, and the ability to re-hydrate in response to erratic precipitation events may contribute to the advantage of one of the two species (Owens and Schreiber 1992; McDowell 2002). Another potential difference is the ability

to tolerate environmental stresses and maintain optimal nutrient sequestration (Aerts et al. 1991). Aerts (1995) called for more research on the ecology of roots of evergreen species to more accurately assess their resource use. The roots of live oak and Ashe juniper penetrate cracks in the underlying limestone of the Plateau and are possibly able to access stored water, which enable the trees to tolerate drought conditions (Jackson et al. 1999).

Because Ashe juniper does not maintain a long-term viable seedbank and does not resprout after disturbance, life history strategies should be discounted as a factor benefiting Ashe juniper establishment and growth over live oak (Blomquist 1990; Owens and Schliesing 1995). However, other factors may be limiting the establishment of live oak, such as preferential grazing by whitetail deer and the suppression of natural fires. Due to their monoterpene content juniper also has the ability to avoid pests and mammalian feeding (Dearing et al. 2000). Miller et al. (1995) suggest that a competitive advantage of juniper may be gained during the early growth stages when it exhibits higher photosynthetic rates than after the transition to the adult scale-like foliages. The ability to keep gas exchange constant at low levels and be less affected by environmental changes may allow Ashe juniper to maintain physiological processes and possibly have a competitive advantage over live oak under prolonged water limited conditions. Kraaij and Cramer (1999) concluded that the invasive potential of one species was unlikely to be completely caused by photosynthetic characteristics. Therefore knowledge of species-specific responses to environmental stimuli and, as suggested by Schwinning et al.

(2004), to ecosystem resource availability pulses, is needed to understand the relative distribution within a plant community.

Canopy characteristics appear to be important to the success of each tree species. Longevity of leaves within the canopy will affect the total carbon gain throughout the year (Herrick and Thomas 2003; Xie and Luo 2003). Oak will replace its leaves more frequently and be more susceptible to pests than juniper, adding to the advantage of Ashe juniper (Owens and Schreiber 1992). Leaf position within the canopy has been shown to affect photosynthesis, transpiration, and conductance in both juniper and oak (Owens 1996). Ashe juniper has a more uniform distribution of carbon uptake (Owens 1996) as well as leaf area distribution, throughout the canopy (Hicks and Dugas 1998) and maintains an overall larger leaf area, total daily carbon gain and water loss (Owens 1996) than oak. Because of the cylindrical shape of the leaves, juniper trees can maintain a larger photosynthetically active leaf area compared to similar sized oak trees (Owens 1996; Hicks and Dugas 1998). Compared to oak, the scale-like leaf structure and large leaf area cause juniper trees to retain more water in the canopy than oak, thereby less precipitation will reach the surface for aquifer recharge (Owens et al. 2006).

To predict future effects of increases of global atmospheric carbon concentrations changes it is necessary to evaluate plant responses under ambient carbon dioxide levels and to assess the extent to which changes in phenology and climate affect carbon uptake and water loss of the Edwards Plateau ecosystems. Therefore it is necessary to conduct long-term studies of net CO_2 exchange of the established plant communities. Further, to understand carbon gain/loss and to understand the mechanisms

regulating Ashe juniper encroachment, the contribution of the dominant woody species to the Edwards Plateau carbon flux have to be determined. I tested the hypothesis that the ability of Ashe juniper to encroach on the Edwards Plateau relies on the ability of the species to maintain physiological processes under adverse conditions and that differences in canopy characteristics between the two dominant tree species adds to this competitiveness. Furthermore, it is of interest to determine if increased tree density alters the water budget of the Plateau as much as it increases possible carbon sequestration. For juniper to be exhibiting its present encroachment as well as oak being able to persist and expand in this area, it would indicate that both species are possibly either employing some drought tolerance mechanism or are able to tap into stored water resources. It may also simply depend on a combination of physiological characteristics of the two species as well as adaptability to environmental conditions. Objectives for this part of the study were to:

1. Compare relative leaf net photosynthesis of Ashe juniper and live oak and relate that to seasonal and environmental influences that may influence species distribution within an oak-juniper system.
2. Scale leaf level data to canopy-level to compare relative contributions of Ashe juniper and live oak trees to ecosystem gas exchange.

Materials and Methods

Site Description

The research was conducted from November 2004 to October 2005, in an Ashe juniper-live oak dominated forest on the Texas State University Freeman Ranch (29.9°9 N, 98° W) on the Edwards Plateau near San Marcos, Texas. The ranch area is classified as juniper-oak savanna (U.S. Soil Conservation Staff 1984). The climate at the site is semi-arid with a bi-modal pattern of precipitation with peaks during the fall and spring. Summers are relatively dry and hot, but may have very intense and scattered rain events. Mean annual precipitation is 857 mm and mean annual temperature in this area is 19.4ºC (Dixon 2000). The year 2004 was exceptionally wet (1779 mm) for this area and 2005 was relatively dry (921 mm) with periods of drought, but still above normal average.

The soil of the juniper-oak forest site is a Comfort, stony clay with rock outcrop complex formed over fractured limestone (U.S. Soil Conservation Staff 1984). In spite of the high clay content, the soils at the Freeman Ranch have limited moisture storage due to the shallowness of the soil and high composition of solid and fragmented rock (Carson 2000). The underlying geology is characterized by fissures, sinkholes, streams and underground caves which in combination allow for rainfall to infiltrate rapidly when it reaches the surface (Owens et al. 2006).

Gas Exchange Measurements

Leaf-level gas exchange measurements were measured with a portable, infrared gas analyzer system (Li-6400, Li-Cor Inc., Lincoln NE, United States) (Figure 1).

Figure 1. Portable open chamber infrared gas analyzer (Li 6400) used for sampling gas exchange of live oak and Ashe juniper.

The system was calibrated at the beginning and end of the season using calibration gasses of known concentration. The span for water vapor was calibrated with a Li-Cor 610 dew point generator (Li-Cor Inc., Lincoln NE, United States). Zeros for both CO_2

and water vapor were set each sampling day using fresh scrub chemicals. Zero and span shifts for both CO_2 and water vapor were negligible throughout the study. The sample trees were located in a dense forest (Fig. 2). Due to canopy structure, gas exchange measurements were made on sun and shade leaves and in two cardinal directions (SE and NW). Fully expanded leaves were subjectively selected employing the criteria of the leaves being 1) fully expanded, 2) located at the outer portions of the branch, 3) being intact and undamaged, and 4) visually undifferentiated from surrounding leaves. Eight trees, four of each species positioned in four pairs, were permanently marked and canopies were accessed via platforms (Fig. 3). The trees were sampled on a monthly basis during the growing season and once every 6 to 8 weeks during the non-growing season (Table 1).

Figure 2. Dense stand of Ashe juniper and live oak at the Freeman Ranch forest site.

Figure 3. Taking measurements at the Freeman Ranch forest site.

Table 1. Sample dates for study of seasonal gas exchange in Ashe juniper and live oak. Fall 2004-2005.

Sample Day	1	67	119	170	214	246	280	325	346
Dates[1]	Nov 9 2004	Jan 15 2005	Mar 8 2005	Apr 28 2005	Jun 11 2005	Jul 13 2005	Aug 16 2005	Sep 30 2005	Oct 21 2005

[1]There was a gap in the spacing of sampling dates between April 28, 2005 and June 11, 2005 due to leaves being eaten by fall webworm *Hyphantria cunea. S*ampling had to be postponed in the fall of 2005 to September 30 due to hurricane threats.

All measurements were at ambient CO_2 and light levels, using the track PAR function and LED light source. Leaves were allowed to stabilize in the leaf chamber before logging. After sampling, all leaves were carefully removed and projected leaf areas were determined using a Li-3100 leaf area meter. They were subsequently dried and weighed for determining specific leaf mass and area. Photosynthesis (A), transpiration (E), and stomatal conductance (g_{sw}) are all expressed on a one-sided leaf area (m^2) basis. Juniper leaf area was adjusted by multiplying the projected area by 0.5π to correct for the cylindrical shape of the needles (Cregg 1991). All gas exchange measurements were made between mid-morning and mid-afternoon. Net assimilation was calculated, using Li-Cor software which employs the following equations:

$$A = \frac{F(C_r - C_s)}{100S} - C_s E \qquad [1]$$

where A is net leaf carbon assimilation rate (μmol CO_2 m^{-2} s^{-1}), F is air flow rate (μmol s^{-1}), C_r and C_s are sample and reference CO_2 concentrations (μmol CO_2 mol^{-1} air), S is measured leaf area (cm^2), and E is transpiration (mmol H_2O m^2 s^{-1}). Transpiration (E) was calculated using the equation

$$E = \frac{F(W_s - W_r)}{100S(1000 - W_s)} \qquad [2]$$

where E is transpiration (mmol H_2O m^{-2} s^{-1}), F is air flow rate (μmol s^{-1}), W_s and W_r are sample and reference water mole fractions (mmol H_2O mol air^{-1}). Stomatal conductance for water vapor was calculated using the equation

$$g_{sw} = \frac{1}{\frac{1}{g_{tw}} - \frac{k_f}{g_{bw}}} \quad [3]$$

g_{sw} is stomatal conductance (mol H_2O m^{-2} s^{-1}), g_{tw} is total conductance of the leaf, g_{bw} is boundary layer conductance, and k_f is a factor based on the stomatal ratio of the leaf. Total conductance to water vapor was calculated by the equation

$$g_{tw} = \frac{E\left(1000 - \frac{W_l + W_s}{2}\right)}{W_l - W_s} \quad [4]$$

where W_l is the molar fraction of water vapor within the leaf (mmol H_2O mol air^{-1}) computed from leaf temperature and total atmospheric pressure.

Leaf Characteristics

Specific leaf area (SLA) (cm^2 g^{-1}) was calculated using one-sided measured leaf area and dry weight of leaves.

Water Use Efficiency

Mean water use efficiency (*WUE*) (μmol $mmol^{-1}$) for each sample was calculated using instantaneous leaf level gas exchange values and the equation

$$WUE = \frac{A}{E} \quad [5]$$

Water Potential

Xylem water potential (Ψ_w) was measured on twigs for each direction and canopy level, using a Scholander pressure chamber. Water potentials were determined immediately before or during the gas exchange sample for each leaf.

Leaf Area Indices

Individual tree canopy area was determined to quantify species contribution to community gas exchange by scaling from leaf to tree level. Leaf area indices were determined using LIDAR imagery supplied by the University of Texas (courtesy of Amy Neuenschwander).

Scaling to Whole-tree Level

To approximate whole-tree canopy gas exchange (*CL*), carbon uptake (μmol CO_2 s^{-1}) and transpirational water loss (mmol H_2O s^{-1}), we located 12 trees on the Freeman Ranch using a sub-meter accuracy global positioning device. Each of the trees was within the

woodland site, but was deliberately selected to not have intermingled canopies of the 2 tree species. From the location, we assumed each tree canopy had a radius of 3 m and Amy Neuenschwander (University of Texas, Center for Space Research) extracted LAI estimates around these points from the LIDAR imagery. LAI values for juniper obtained from LIDAR were adjusted by 0.5π to correct for the cylindrical shape of the needles (Cregg 1991). LAI for oak was assumed to be half shade and half sun because of differences in productivity between canopy levels, whereas juniper showed very little difference between canopy levels and a more even distribution of gas exchange (Owens 1996) and leaf area (Hicks and Dugas 1998). Therefore, juniper LAI was not divided between sun and shade. Whole-tree gas exchange for juniper (CL_j) and oak (CL_o) was determined using the equations

$$CL_j = (R_{\text{shade+sun}} \cdot LAI) \cdot CA \quad [6]$$

$$CL_o = ((R_{\text{shade}} \cdot 0.5\text{LAI}) + (R_{\text{sun}} \cdot 0.5LAI)) \cdot CA \quad [7]$$

The rate R is the mean net photosynthesis (μmol CO_2 m^{-2} s^{-1}) or transpiration (mmol H_2O m^{-2} s^{-1}) rate of the individual trees. CA is the total canopy area, assumed to be a circle with radius r = 3 m (28.274 m^2). LAI is leaf area index. Canopy level values were calculated for each tree for each sample date.

Data Analysis

All variables were initially tested in a multifactor analysis of variance. We used repeated measures analysis of covariance with an autoregressive covariance structure to test for differences in gas exchange, stomatal conductance, water potential, and water use efficiency (Statistical Analysis System ver. 9.0, SAS Institute Inc. Cary, NC, USA). Individual trees were nested within species to compare the differences within (Sun leaf vs. Shade leaf) and between species for all physiological data at $P < 0.05$. Leaf water potential and PAR were used as covariates. Canopy-level means and standard errors were calculated for direct comparisons.

Results

Leaf Characteristics

Mean specific leaf area (SLA) was greater for live oak than for Ashe juniper (49.76 ± 0.750 vs. 32.71±0.366 $cm^2\ g^{-1}$, throughout the study (Table 2). There were no significant differences in either SLA based on canopy location (levels or direction) for either species. Values of SLA were stable throughout the year and oak consistently maintained a higher area to mass ratio. It must be noted that values for juniper SLA of the first sampling date deviated substantially from the rest and has been omitted. This was most likely due to personal errors when measuring the leaves.

Table 2. Specific leaf area (SLA) (cm^2 g^{-1}) sample averages (±SE) for Ashe juniper and live oak.

Date	Nov 9 2004	Jan 15 2005	Mar 8 2005	Apr28 2005	Jun 11 2005	Jul 13 2005	Aug16 2005	Sep 30 2005	Oct 21 2005
Sample	1	67	119	170	214	246	280	325	346
Oak	44.65	43.34	47.00	52.79	60.11	51.60	51.25	51.43	46.73
	±2.46	±2.13	±1.74	±2.50	±1.67	±1.75	±1.45	±1.70	±1.70
Juniper	-	30.87	29.83	31.77	35.03	31.30	32.94	35.29	34.55
	-	±1.32	±1.10	±0.73	±0.73	±0.85	±0.84	±0.93	±0.92

Water Potential

The mean (±SE) water potential for juniper ranged from –3.5 (±0.13) MPa in July of 2005 to -1.3 (±0.05) MPa in November 2004. For oak, water potentials ranged from -1.4 (±0.06) MPa in November of 2004 to -2.50 (±0.18) MPa in October of 2005. During the first half of the sampling period, oak was under greater water stress than juniper; however, by July 2005 juniper began to experience lower water potentials. Precipitation in the fall of 2004 was higher, well above normal, than during the sampling period in 2005 where the trees experienced periods of severe drought, especially towards the end of the summer and during the fall (Fig.4). Throughout the sampling period, the two tree species had different patterns of water stress (Fig. 5, $P < 0.0001$).

During the early part of the study, water potential was similar between sun and shade leaves, but by the latter portion of the period leaves in the sun were experiencing greater water stress (Fig. 6, $P = 0.049$). The canopy direction did not affect water potentials of either sun or shade leaves ($P = 0.8653$). The mean water potentials for leaves in the shade ranged from -2.8 (±0.19) MPa in July 2005 to -1.3 (±0.06) in November 2004, and for sunlit leaves from -2.9 (±0.19) MPa in July 2005 to -1.4 (± 0.05) MPa in November 2004 (Fig. 6). The most pronounced differences occurred in August, September, and October of 2005.

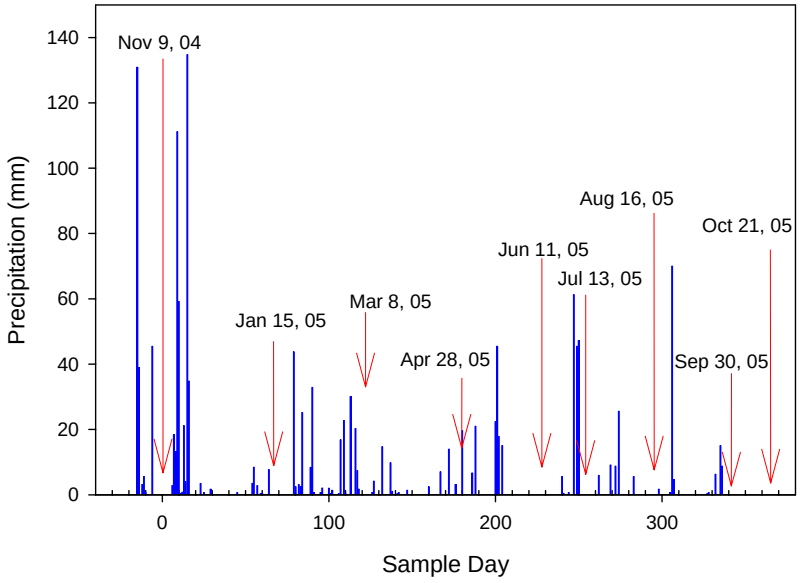

Figure 4. Precipitation (mm) throughout the sampling period. Arrows indicate dates on which gas exchange measurements were made.

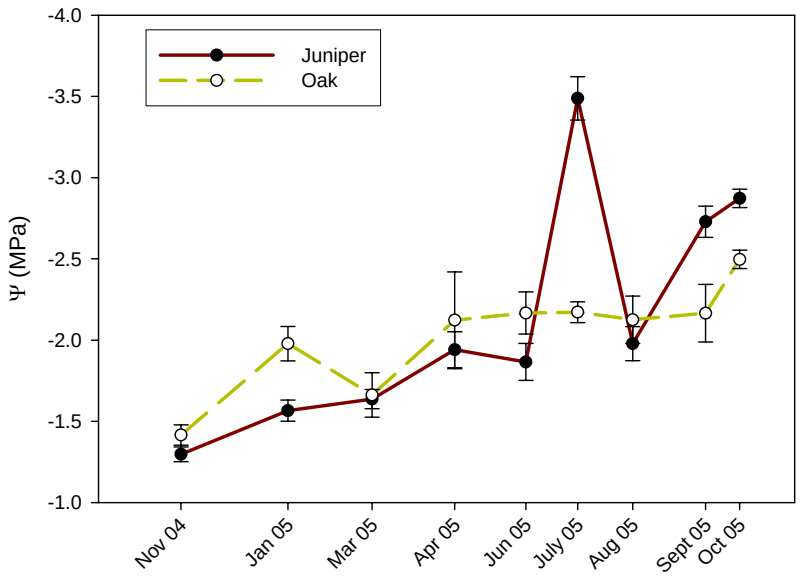

Figure 5. Xylem water potentials of live oak and Ashe juniper in a forest setting over the course of the study. Error bars represent ± SE.

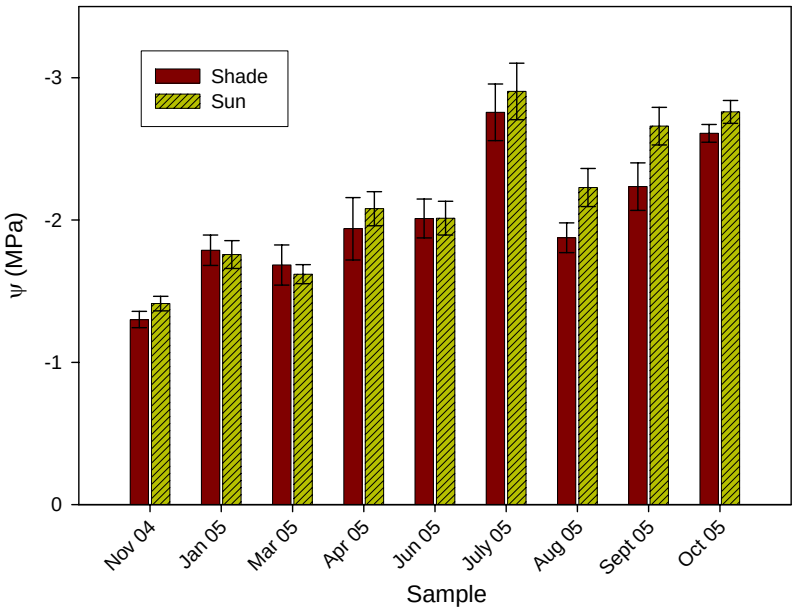

Figure 6. Xylem water potentials of shade and sunlit leaves of both live oak and Ashe juniper over the course of the study. Error bars represent ± SE.

Photosynthesis

Species carbon assimilation rates were greater ($P < 0.001$) for oak than for juniper (13.12 ±0.6 vs. 6.47 ±0.4 μmol CO_2 m^{-2} s^{-1}, respectively) (Fig. 7). Ashe juniper consistently exhibited lower seasonal flux and carbon assimilation rates than live oak. The two species demonstrated different rates throughout the sampling period ($P = 0.0270$), photosynthesis was lowest in October for both species (2.47 for juniper and 6.46 for oak μmol CO_2 m^{-2} s^{-1}) and greatest in November 2004 for juniper (13.02 μmol CO_2 m^{-2} s^{-1}) and April 2005 for oak (21.64 μmol CO_2 m^{-2} s^{-1}). Leaf position within the tree canopy affected carbon assimilation differently in both species ($P = 0.0066$). There was no significant difference in carbon assimilation between sunlit and shade leaves for juniper trees, but sunlit leaves of oak trees had significantly greater assimilation rates (Fig. 8).

At onset of new leaf growth in April, live oak exhibited a surge in carbon assimilation not expressed to a similar extent by juniper (Fig 7). At the end of the sampling period in October 2005 both species showed much lower photosynthetic rates compared to November 2004, where rainfall was higher (Fig. 8). There was no significant influence on photosynthesis of leaf direction in canopy ($P = 0.3294$).

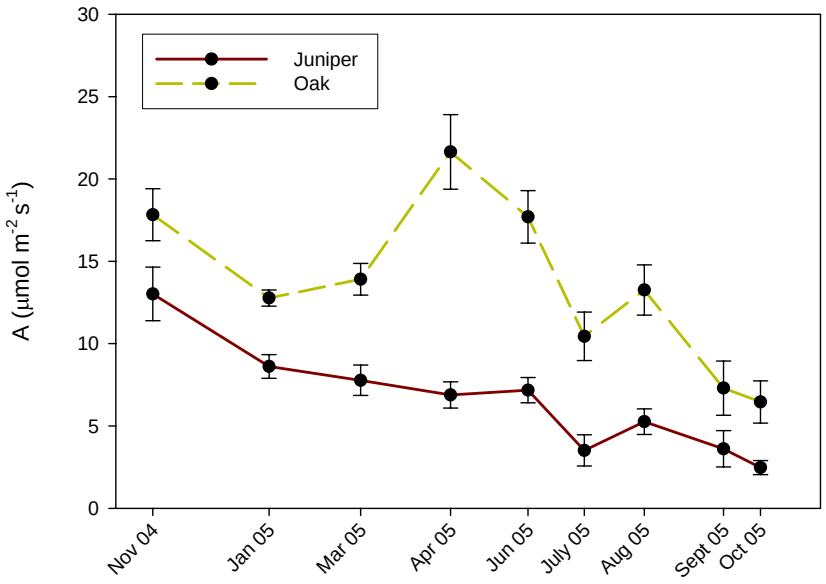

Figure 7. Mean leaf level net photosynthesis of live oak and Ashe juniper over a 1 year period. Trees were growing in a forest setting. Error bars represent ± SE.

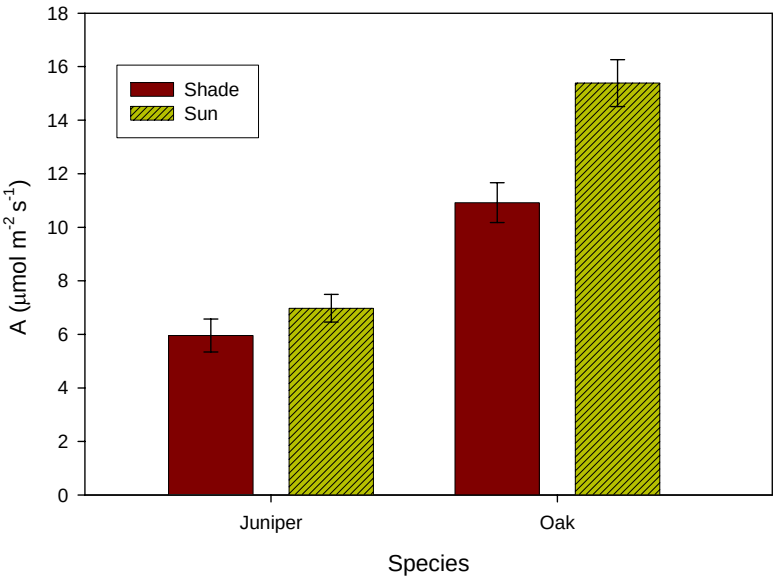

Figure 8. Comparison of net photosynthesis of shade and sunlit leaves. Error bars represent ± SE.

Transpiration

Oak consistently had greater rates of transpiration at each sample date and exhibited greater fluctuation between sample dates than juniper (Fig. 9, $P < 0.0001$). The minimum transpiration rate (±SE) for Ashe juniper was 0.5 (±0.1) in September 2005 and the maximum was 2.3 (±0.3) (mmol H_2O m^{-2} s^{-1}) in June of 2005. Live oak exhibited the lowest average transpiration, 1.2 (±0.3) in September 2005 and highest level, 6.7 (±0.7) (mmol H_2O m^{-2} s^{-1}) in June 2005. Leaf level position in the canopy affected transpiration levels ($P = 0.0035$). Sunlit leaves of both oak and juniper had greater levels of transpiration, 4.0 (±0.3) and 1.4 (±0.1) (mmol H_2O m^{-2} s^{-1}) respectively, compared to 3.0 (±0.3) and 1.2 (±0.1) (mmol H_2O m^{-2} s^{-1}) of oak and juniper shade leaves. The differences between canopy levels (Fig. 10) of both species were, as the case was with photosynthesis, more apparent between live oak leaves. Effects of water status of the trees (Ψ_w) on transpiration were marginally insignificant ($P = 0.069$). Overall, live oak appears to have higher rates of instantaneous, leaf-level water loss than juniper.

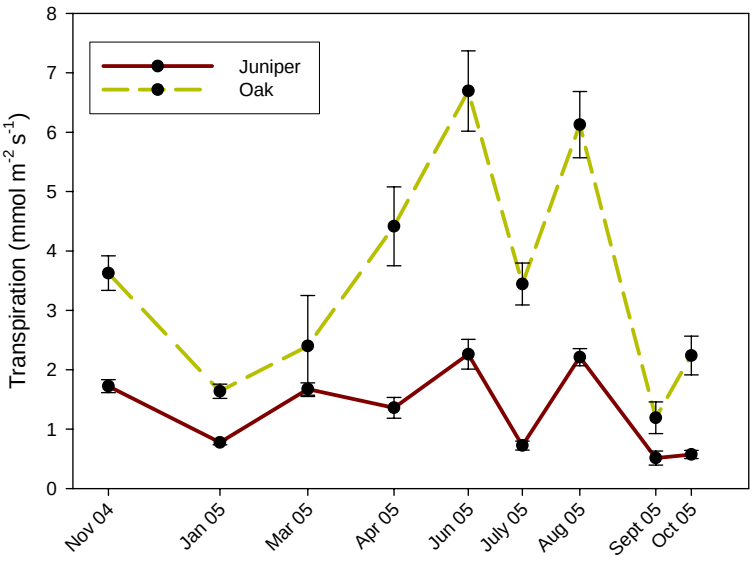

Figure 9. Mean leaf-level transpiration of juniper and oak over a 1 year period. Trees were growing in a forest setting. Error bars represent ± SE.

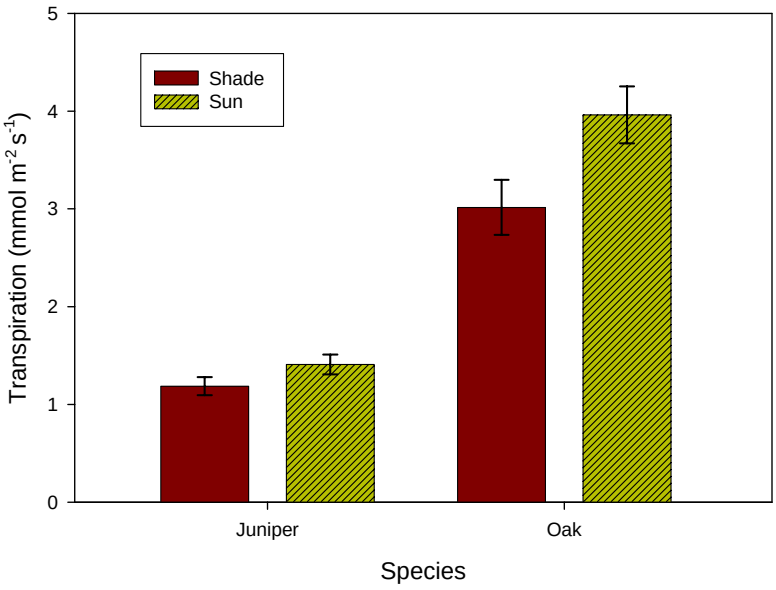

Figure 10. Transpiration of shade and sunlit leaves. Error bars represent ± SE.

Conductance

Mean conductance values were greater ($P < 0.0001$) for oak than juniper (0.18 ± 0.01 vs. 0.06 ± 0.01 mol H_2O m^{-2} s^{-1}, respectively). Throughout the year, Ashe juniper consistently exhibited lower conductance and less seasonal variability than live oak (Fig. 11). The lowest levels were in October 2005 for both species (0.08 ± 0.01 vs. 0.02 ± 0.00 mol H_2O m^{-2} s^{-1} for oak and juniper), which is when photosynthesis and precipitation were also at their lowest (compare with Figs.5 and 8). The greatest conductivity occurred in November 2004 for both oak and juniper (0.27 ± 0.03 vs. 0.10 ± 0.01 mol H_2O m^{-2} s^{-1}, respectively). Throughout the course of the study, live oak never reached the low conductance values commonly observed for Ashe juniper. There was no significant influence of leaf level ($P = 0.9422$) or direction ($P = 0.9211$) on conductance values for either species.

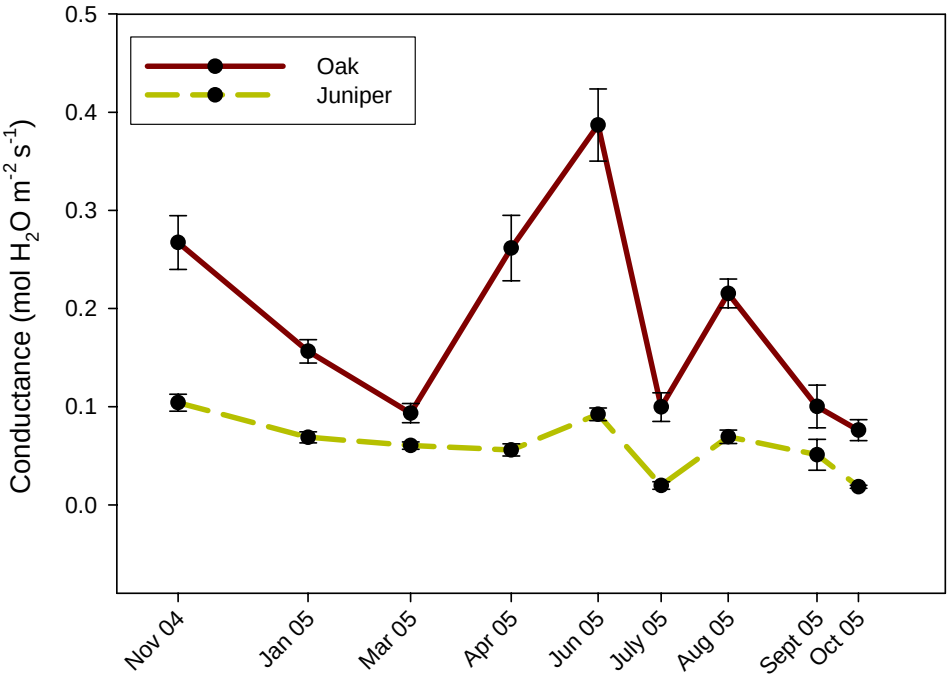

Figure 11. Mean stomatal conductance for juniper and oak during a 1 year period. Error bars represent ± SE.

Water Use Efficiency

The mean water use efficiency (WUE) for both species (Fig. 12) was slightly greater (P = 0.0370) for juniper 5.88 (±0.25) μmol $mmol^{-1}$ than oak 4.88 (±0.41) μmol $mmol^{-1}$). Leaf canopy direction affected WUE differently (P = 0.0012) in the two species (Fig. 13). WUE for northwest leaves was 4.88 (±0.43) μmol $mmol^{-1}$ and WUE for southeast leaves was 5.17 (±0.39) μmol $mmol^{-1}$. Southeast WUE was 6.86 (±0.68) μmol $mmol^{-1}$ for juniper and 4.61 (±0.31) μmol $mmol^{-1}$ for oak. Live oak WUE did not differ significantly with direction, but juniper did, with WUE being greater on the southeast side of the canopy. WUE between species differed significantly (P = 0.0454) throughout the sampling period and was also affected by leaf canopy direction but in an unpredictable manner throughout the study (Fig 14). Northwest directed leaves had the lowest WUE in August 2005 (2.25 ±0.29 μmol $mmol^{-1}$) and greatest in January 2005 (10.09 ±0.76 μmol $mmol^{-1}$). For the southeast directed leaves the minimum was also in August 2005 (2.53 ±0.35 μmol $mmol^{-1}$) and also greatest in January 2005 (9.33 ±0.88 μmol $mmol^{-1}$).

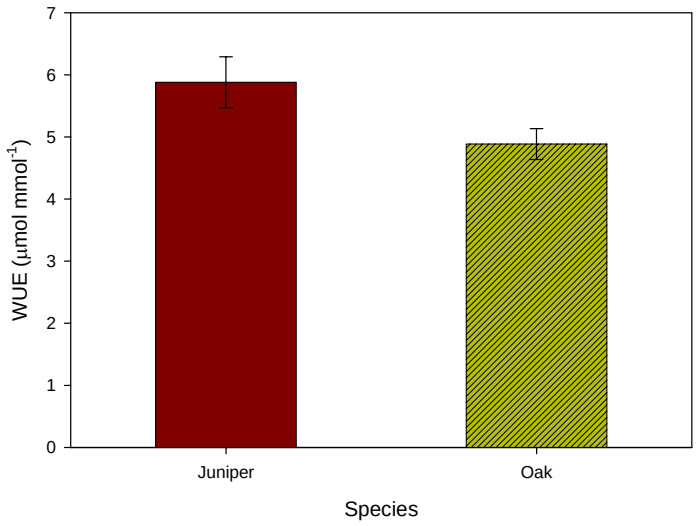

Figure 12. Mean water use efficiency for Ashe juniper and live oak in a forest setting. Error bars represent ± SE.

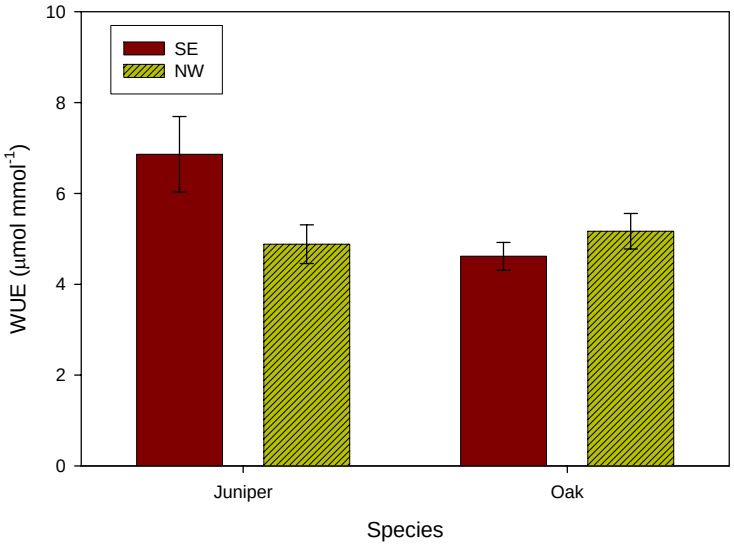

Figure 13. Mean water use efficiency for Ashe juniper and live oak by cardinal direction and species. Error bars represent ± SE.

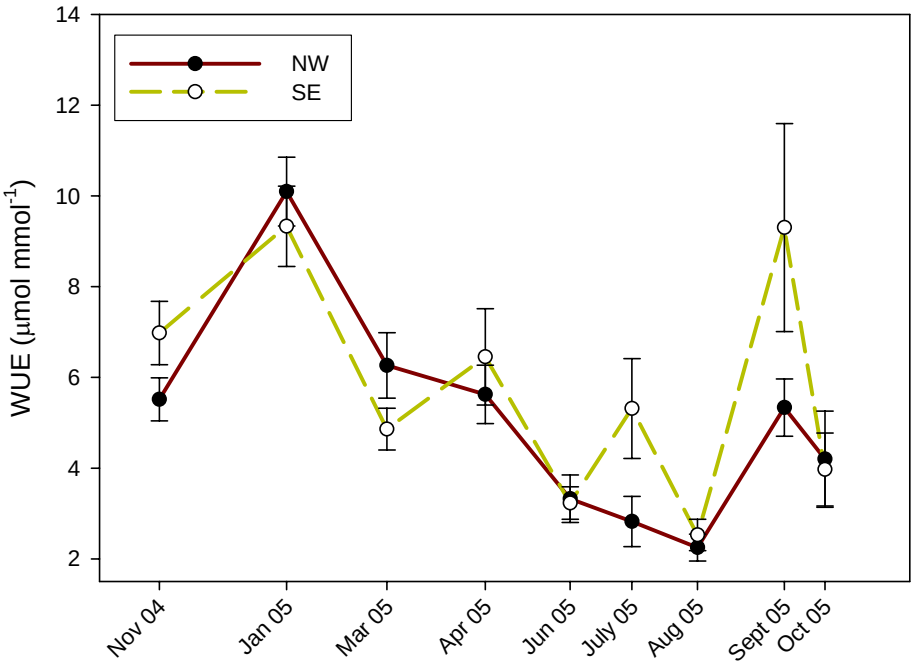

Figure 14. Water use efficiency for northwest (NW) and southeast (SE) directions, throughout a 1 year period. Error bars represent ± SE.

Scaling to Whole-tree Level

The results of my scaling of leaf-level measurements to whole-tree level, estimated the annual mean (±SE) carbon uptake to be 263.13 (±22.69) μmol s^{-1} for Ashe juniper and 439.74 (±16.16) μmol s^{-1} for live oak. Transpirational water loss was twice as much for live oak (113.67 ± 21.07 mmol s^{-1}) as for Ashe juniper (52.85 ± 9.16 mmol s^{-1}) as averaged among trees throughout the year. The maximum canopy CO_2 gain occurred in November 2004 for both species (528.65 ±111.52 for juniper and 729.55 ± 85.81 μmol s^{-1} for oak) (Fig.15). Minimum was 100.34 (±21.17) and 209.10 (±24.59) μmol s^{-1} for juniper and oak, respectively. Both occurred in October 2005. Transpirational water loss was greatest for both juniper and oak (91.79 ±19.36 and 216.79 ±25.50 mmol s^{-1}, respectively) in June 2005, and least in September 2005 (20.79 (±4.39) for juniper and 38.57 (±4.54) for oak (Fig. 16). Canopy-level results did not deviate from the same general pattern as leaf level measurements described.

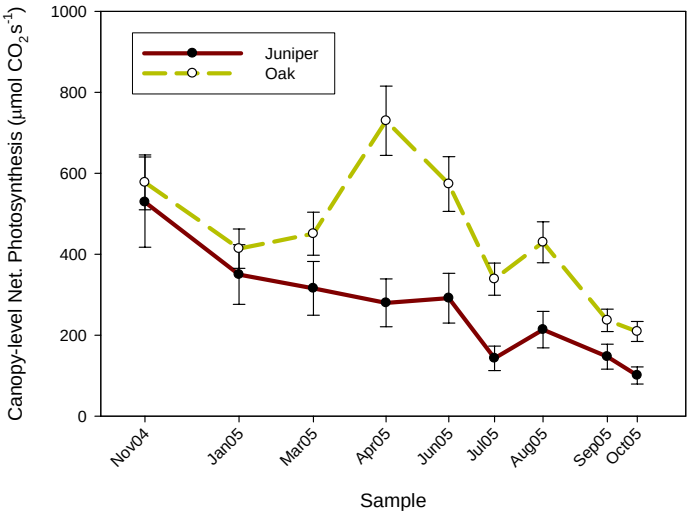

Figure 15. Mean whole-tree CO_2 gain for juniper and oak in a forest setting. Error bars represent ± SE.

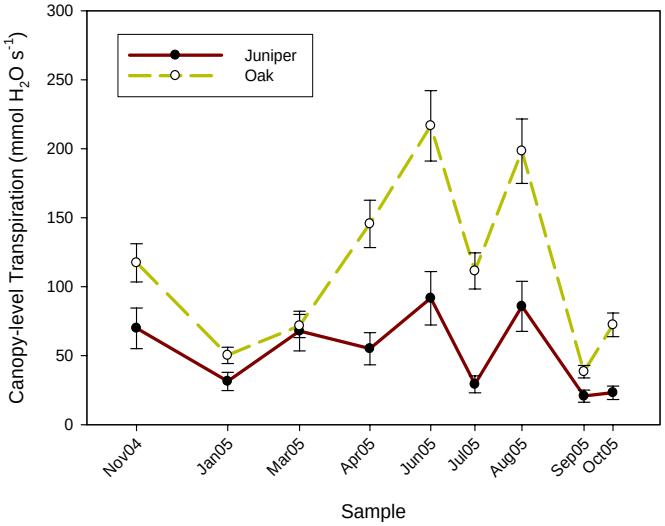

Figure 16. Mean whole-tree transpirational water loss for juniper and oak in a forest setting. Error bars represent ± SE.

Discussion

When comparing the xylem water potentials at the forest site to the precipitation distribution throughout the sampling period, it appears that tree water status is influenced by the amount of rain received. The high amounts of rain preceding the November 2004 data collection resulted in the least negative Ψ_w for the sampling period. The values steadily became more negative towards the fall of 2005 (Fig. 5 and Fig. 6). Effects of rainfall depended on both amount of rain received as well as distribution of precipitation events. This would disagree with the hypothesis of some of the adaptation mechanisms in semi-arid shrubs proposed by Seyfried et al. (2005) which minimizes reliance on rainfall and seasonal fluctuation of water potentials. The few intense rainfall events during June and July 2005 (Fig. 4) were not sufficient to prevent Ashe juniper from reaching the lowest average Ψ_w (-3.5 ± 0.1 MPa) measured throughout this study. These low values coincided with decreased precipitation during the latter part of the study (Fig. 4), suggesting that oak in this area is less affected by erratic distribution and amount of rainfall whereas, Ashe juniper appeared to be more dependent on rain events and readily available soil moisture to maintain its water status, than previously described for other areas of the Edwards Plateau by Owens and Schreiber (1992). There are physiological and morphological characteristics of the live oak trees that may explain this difference. Jackson et al. (1999) found that live oak was more deeply rooted than juniper in parts of the Edwards Plateau and can access deeper sources of soil water. Live oak also maintains constant xylem vessel conductance and pit membrane pore diameters between

roots and stems, without becoming more susceptible to cavitation (McElrone et al. 2004), thereby offering oak on the Edwards Plateau an advantage during droughts. Epron and Dreyer (1993) investigated how two co-existing oak species were affected by prolonged drought in a natural environment and a similar study was conducted by Xu and Baldocchi (2003). They concluded the rather high tolerance to drought was most likely due to deep rooting structures or better water use efficiency, rather than physiological or morphological leaf differences (Epron and Dreyer 1993). Physiological parameters respond in parallel to seasonal and environmental changes, in particular decline in soil water and temperature differences (Xu and Baldocchi 2003).

Only in August and September of 2005 were there significant differences in Ψ_w between shade and sunlit leaves (Fig 6). All the trees grow in close proximity and do not develop extensive canopies with multiple levels. The tree canopy is shell-like and sunlight is easily distributed throughout the canopy. Whether or not it is appropriate to speak of sun and shade leaves is questionable, since the physical distance between the two layers is minimal. The differences between Ψ_w of sun and shade levels appearing towards the end of the sampling period may be caused by available water being so scarce that the higher levels of gas exchange in sun leaves, especially in live oak, become important (Figs. 8 and Fig 10).

The measurements of specific leaf area (SLA) showed no differences between shade and sun leaves, which differs from studies of co-occurring species in similar environments (Hamerlynck and Knapp 1994; Taiz and Zeiger 2002). The lack of any

difference in SLA between sunlit and shade leaves would support the observation of the thin canopy not allowing for sun and shade leaves characteristics to develop.

The gas exchange data showing that photosynthesis and transpiration differed between canopy layers support those of Owens (1996) who showed that transpiration and photosynthesis of both species decreased from top to bottom of canopies, more so in live oak than Ashe juniper. The trends of photosynthesis and transpiration between sampling dates, showed both evergreen species to be affected by precipitation amounts. This is in agreement with the results found on same species by Owens and Schreiber (1992) where net photosynthethic rates followed precipitation distribution. However, our results are also dissimilar from that study. I found oak to be able to maintain photosynthesis at higher levels than juniper even when precipitation was limited. Owens and Schreiber (1992) found there to be a significant relationship between oak photosynthesis and rainfall, but a less dramatic effect on juniper photosynthesis and designated this species as more drought adapted/tolerant than live oak.

Differences in internal structure and hydraulic properties of the roots and ability to tap into stored water (Jackson et al. 1999; McElrone et al. 2004) may have contributed to the success of one species over the other (Bowman and Roberts 1985). In addition, differences between this and previous results could be attributed to dissimilarities in stand structure of the two studies. This study was in a dense forest, whereas the study by Owens and Schreiber (1992) was in a more open area. Trees were growing separately and had fully expanded canopies.

Leaf level flux differences between live oak and Ashe juniper in this study showed oak to have the greater variability than juniper, and photosynthetic rates of oak were double those of juniper. Owens and Schreiber (1992) and Owens (1996) found instantaneous and mean photosynthetic rates of live oak to be three to four times greater than those of Ashe juniper, but Ashe juniper typically had a much greater canopy level carbon uptake because of its greater leaf area (Owens 1996). Unlike Owens (1996) I did not find gas exchange to vary with leaf cardinal direction in either species. This may be attributed to dissimilar tree characteristics of the two studies.

Maximum net photosynthesis for oak occurred during spring and summer followed by a decrease in the fall, and the highest rates of transpiration occurred during mid-summer. As with Miller et al. (1992) and Owens and Schreiber (1992), juniper showed limited fluctuation in gas exchange, in relation to time of year or new leaf growth. Miller et al. (1992) found photosynthetic fluxes and responsiveness to environmental stimuli to be reduced significantly in adult junipers.

Live oak maintained greater stomatal conductance throughout the year compared to Ashe juniper, even under periods of stress. Conductance followed the course of photosynthesis and transpiration closely for both species. Similarly reported by Owens and Schreiber (1992), but I cannot reach the same conclusion, that the average low stomatal conductance demonstrated by Ashe juniper necessarily makes this species more drought tolerant, based on the low water potentials and net photosynthesis exhibited by juniper. Studies by Oliveira et al. (1996) in cork oak, on the Iberian Peninsula showed significant differences in conductance within canopies between canopy height and

direction of leaf position, under ambient conditions. Most significant was the effect of leaf direction rather than height. However, these interactions were most pronounced during the spring, when growth was most intensive (Oliveira et al. 1996).

My study indicated that Ashe juniper had slightly higher WUE than live oak, opposing the findings of Owens and Schreiber (1992). Surprisingly, even though measurements of photosynthesis and transpiration showed no differences between leaf directions, WUE did. Main differences were in juniper, showing greater WUE in south-east directed leaves. The standard error for the southeast leaves of juniper was also much greater than northwest juniper and both directions in oak. Throughout the year leaf canopy direction was shown to be statistically significant in affecting WUE, but comparing this to Fig.14, neither southeast nor northwest consistently exhibited the greater or lesser water-use efficiency. It must be concluded that leaf placement in the canopy does not affect water-use efficiency in a predictable manner. Based on the leaf level results, it is not surprising that our whole-tree level estimates would indicate that in similar sized oak and juniper trees, oak loses approximately twice as much water through transpiration compared to juniper, and also takes up slightly less than twice as much carbon as juniper. These results contrast those of Owens (1996), which estimated that daily carbon gain and water loss for Ashe juniper were significantly greater than for live oak. Environmental and structural tree differences between the two studies may be significant in causing these differences.

Kraaij and Cramer (1999) investigated mechanisms enabling species to invade in a semi-arid environment. They concluded that photosynthetic characteristics are

probably only partially responsible for invasive potential. Light and temperature influenced responses of two co-existing oak species indicated that of the species specific tolerances it was the differences in high tolerance of temperature as well as drought, rather than leaf level photosynthesis and responses to light, that influenced the distribution patterns of these species (Hamerlynck and Knapp 1994). My evaluation of leaf level responses of the two dominant species on the Edwards Plateau did not indicate that Ashe juniper dominates live oak in photosynthetic performance, water use efficiency, or in responses to environmental stimuli. Rather, as suggested in previous studies (Owens 1996; Kraaij and Cramer 1999), it is probably a combination of multiple key factors such as environmental variability, species structural differences, ability to take up and store resources and last but not least, grazing pressure, seedling survival, and suppression of natural fires.

CHAPTER III

LEAF LEVEL LIGHT RESPONSES

Introduction

Measuring long term carbon uptake and whole ecosystem energy flux is important to understand how natural systems are affected by alterations of the environment. Equally important are measurements at smaller scales, such as the leaf-level, to make species specific comparisons. In the previous chapter one of the objectives was to determine the degree to which leaf position within the canopies of Ashe juniper and live oak or environment affects gas exchange rates. The results indicated that differences in productivity between leaf positions were small and mostly depended on environmental variables. I hypothesized that variation among leaves within the canopy was related to amount of light incident on the leaves. This study focuses on the differences in light responses between Ashe juniper and live oak leaves at multiple canopy levels and cardinal directions, as an estimate of physiological capacity. Taiz and Zeiger (2002) established that light compensation and saturation points vary between species and are highly affected by the environment in which leaves are developed. Lewis et al. (1999) developed light response curves of Douglas-fir seedlings under elevated CO_2 and temperature, and showed that leaf position and orientation within the canopy affected responses to elevated carbon dioxide. Several models have attempted to explain

the photosynthethic distribution within a canopy. In all attempts the driving variable was considered to be leaf area index (LAI) as well as light diffusion through the canopy (Kull et al. 1999), rather than variation in photosynthetic capacity. Numerous studies have investigated the relationship between canopy production and light interception (Chen et al. 1994). Takaneaka (1994) showed that branching and canopy growth can be controlled by, and will differ with, amount of light intercepted and the resulting photosynthetic production. Kull et al. (1999) proposed that their modeling efforts support the theory of a steady-state canopy where equilibrium is reached between the differences in productivity among the lower and upper canopy levels, this supports the hypothesis that there is within canopy variation in light responses as well as photosynthetic capacity. Pachepsky and Acock (1996) and Rayment et al. (2000) acknowledged that it is difficult to accurately predict leaf level behavior because multiple factors play a role in controlling physiological processes, such as an increase in atmospheric CO_2, water availability, or temperature.

Leaf-level light and temperature responses have been compared in other co-occurring species where the environment is limited in resources or where there is significant competition among species (Hamerlynck and Knapp 1994). In most studies, the photosynthetic parameters under investigation are instantaneous gas exchange, stomatal conductance, temperature interaction and water use efficiency such as in Owens (1996) or Oliveira (1992). The number of projects that have targeted differences in light saturation and light compensation within canopies and between species are few. To thoroughly understand the dynamics of the juniper-oak ecosystem on the Edwards

plateau, it is necessary to investigate physiological differences between the two species. I hypothesize that leaf responses to light, and photosynthetic capacity, measured by light compensation and light saturation levels, depend upon the position of the leaf within the canopy, and differ between Ashe juniper and live oak. The objective of this study was to determine the degree to which canopy leaf position plays a role in regulating leaf-level light responses of the two site dominant tree species, Ashe juniper and live oak.

Materials and Methods

Site Description

This study was conducted from May 2005 to January 2006 on the Texas State University Freeman Ranch (29.9°9 N, 98° W). The ranch area is classified as juniper-oak savanna (U.S. Soil Conservation Staff 1984). The climate at the site is semi-arid with a bi-modal pattern of precipitation with peaks during the fall and spring. Summers are relatively dry and very hot, but may have very intense and scattered rain events. Mean annual precipitation is 857 mm and mean annual temperature in this area is 19.4ºC (Dixon 2000). The year 2004 was exceptionally wet for this area and 2005 was relatively dry.

The soil of the juniper-oak forest site is a Comfort, stony clay with rock outcrop complex formed over fractured limestone (U.S. Soil Conservation Staff 1984). In spite

of the high clay content, the soils at the Freeman Ranch have limited moisture storage due to the shallowness of the soil and high composition of solid and fragmented rock (Carson 2000). The underlying geology is characterized by fissures, sinkholes, streams and underground caves which in combination allows for rainfall to move rapidly through the ground layers when it reaches the surface (Owens et al. 2006).

Gas Exchange Measurements

All gas exchange measurements were made with a Li-6400 (Li-Cor Inc, Lincoln NE, United States) infrared gas analyzer on May 20 and August 18 2005, and January 13, 2006 to account for possible seasonal differences. The system was calibrated at the beginning and end of the season using calibration gasses of known concentration. The span for water vapor was calibrated with a Li-Cor 610 dew point generator (Li-Cor Inc, Lincoln NE, United States). Zeros for both CO_2 and water vapor were set before each sampling day using fresh scrub chemicals. Zero and span shifts for both CO_2 and water vapor were negligible throughout the study. The sample trees were isolated in an open savanna surrounded by more dense vegetation. Six trees, three of each species, were selected within a 250 m radius. The trees were selected in pairs growing adjacent to one another, resulting in three replications. Sampling was conducted in the spring after new leaves were fully expanded, in mid-summer, and in the winter immediately prior to leaf fall. Measurements were taken at three canopy levels (top, middle, bottom) and in two cardinal directions (SE and NW). Fully expanded leaves were subjectively selected employing the criteria of the leaves being 1) fully expanded, 2) located at the outer

portions of the branch, 3) intact and undamaged, and 4) visually undifferentiated from surrounding leaves. Measurements were conducted with ambient CO_2 at six controlled light levels, starting with the highest values (2000, 1000, 500, 250, 125, and 62 $\mu mol/m^2/s$) using the attached LED light source on the Li-6400 (Li-Cor, Inc, Lincoln, NE). Sampling began with the highest light level and proceeded sequentially to the lowest light level, allowing gas exchange rates to stabilize at each light level before recording the data.

After sampling, all leaves were carefully excised and projected leaf areas were determined using a Li-3100 leaf area meter (Li-Cor Inc, Lincoln NE, United States). Leaves were subsequently dried and weighed to determine specific leaf mass and specific area. All photosynthetic variables measured with the Li-6400 were expressed on a leaf area basis. Juniper leaf area was adjusted by multiplying the projected area by 0.5π to correct for the cylindrical shape of the needles (Cregg 1991). Sampled data was recomputed based on the actual leaf area in the leaf chamber, using Li-Cor software. All gas exchange measurements were made between mid-morning and mid-afternoon. Tree canopy levels were accessed using a trailer mounted boom (Fig 17).

Figure 17. Data collection on savanna trees. Tree canopies were accessed with a trailer mounted boom.

Leaf Characteristics

Specific leaf area (SLA) ($cm^2\ g^{-1}$) was calculated using one-sided measured leaf area and dry weight of leaves.

Water Potential

Xylem water potential (Ψ_w) was measured on twigs for each direction and canopy level sampled, using a Scholander pressure chamber. Water potentials were determined immediately before or during the gas exchange sample for each leaf.

Data Analysis

Light curves were used to calculate light compensation points and light saturation for each sample. The Photosyn Assistant software (Dundee Scientific, Ltd, Dundee, United Kingdom) was used to analyze the photosynthetic responses by use of a non-rectangular hyperbola (Equation 7) according to the model expressed by Prioul and Chartier (1977).

$$A = \frac{o.Q + A_{\max} - \sqrt{(o.Q + A_{\max})^2 - 4.o.Q.k.A_{\max}}}{2k} - R_{day} \qquad [7]$$

where net photosynthesis A is computed from the light level Q, the apparent quantum efficiency o, A_{max} is the light saturated maximum photosynthesis for the upper asymptote, k is a curve convexity factor, and R_{day} is day-time respiration. Figure 18 provides examples of light curves produced with the Photosyn Assistant software, using data collected with the Li-6400.

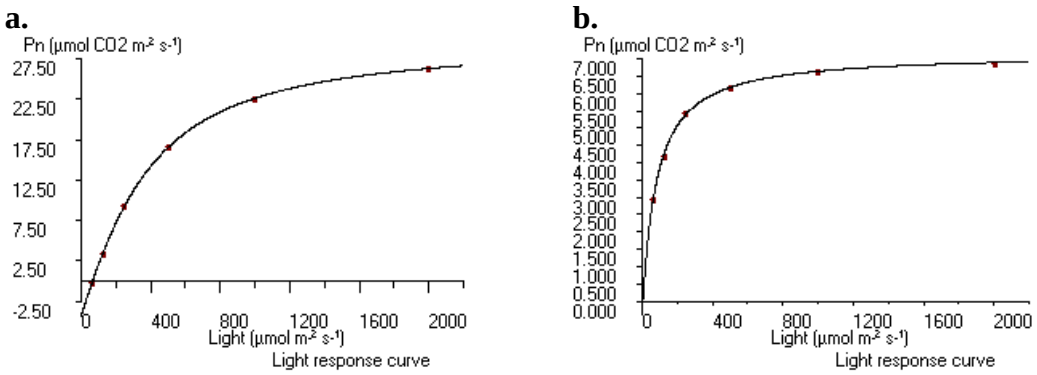

Figure 18. Examples of Photosyn Assistant light response curves as they appear in the program. Computed with data collected using the Li-6400: a. Live oak, August 18, 2005 b. Ashe juniper, August 18, 2005. P_n is net photosynthesis.

Analysis of variance was used to analyze effects of the dependent variables (canopy level, canopy direction, and plant species) on the independent variables of light compensation and light saturation. The original intent was to analyze the data for differences among the three seasonal samples, but this was not possible because of severe water stress in juniper trees in January 2006. A two-sample t-test was used to test for differences in light compensation and light saturation for leaves in different canopy positions for the oak trees in January. Differences between means were tested with Fisher's LSD where appropriate. Water potential and specific leaf area were tested using one-way analysis of variance. Due to limited sample size and the nature of this and other field studies, it is appropriate to accept a lower level of statistical significance ($P < 0.1$) relative to research where all aspects of the environment are controlled.

Results

Leaf Characteristics

Mean specific leaf area (SLA) for juniper and oak (±SE) of the savanna trees were 29.41 (±0.56) and 55.56 (±2.07) g cm^{-2}. The month of observation affected SLA for the two species differently ($P < 0.0001$, Fig. 19). The highest juniper SLA (27.78 ± 0.88 g cm^{-2}) was in May 2005 while oak had its highest SLA (71.43 ± 3.34 g cm^{-2}) in the same month. Differences between canopy levels were also statistically different ($P = 0.0008$, Fig. 20). Fisher's LSD demonstrated a difference between top and bottom levels, but not between top and middle or middle and bottom canopy levels.

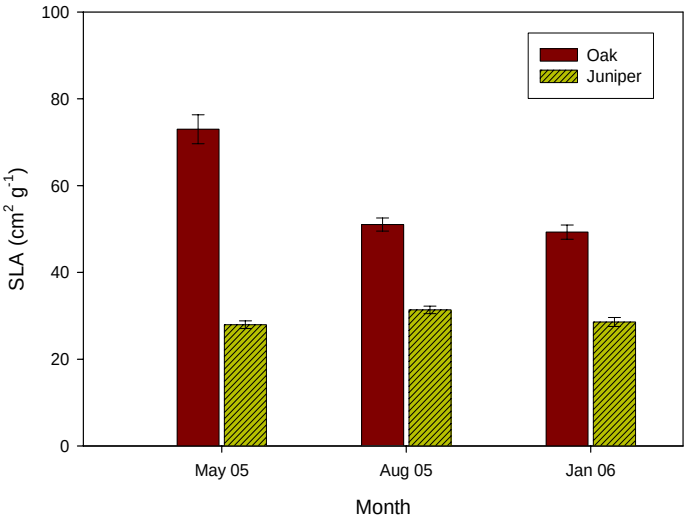

Figure 19. Specific leaf area (SLA) for savanna trees. Measured at three sampling dates. Error bars represent ± SE.

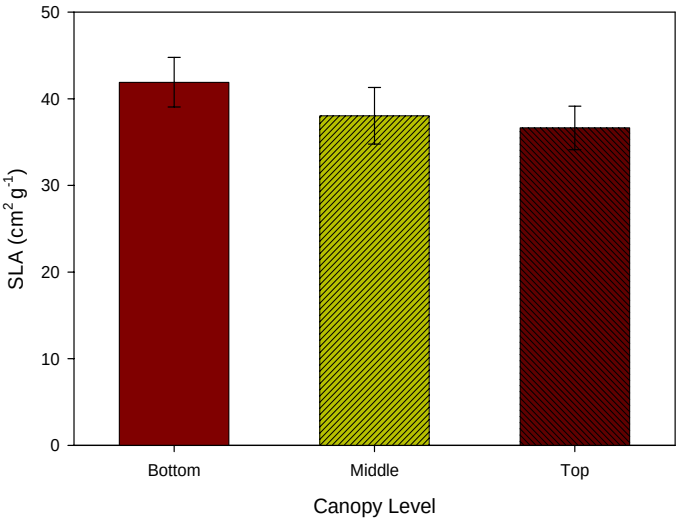

Figure 20. Specific leaf area (SLA) for savanna trees by canopy level. Error bars represent ± SE.

Water Potential

For all three sampling dates xylem water potentials differed significantly between species and among months ($P < 0.0001$). Ashe juniper consistently exhibited lower water potentials than live oak ($P < 0.0001$, Fig. 21). When precipitation was the least (January 2006), this difference was highly significant. The lowest values (±SE) for both oak and juniper were in January 2006 (-2.6 ±0.1 and -4.9 ±0.2 MPa, respectively) during the driest period of this study. The least negative xylem water potentials for both species were in May 2005 (-1.3 ±0.1 and -1.7 ±0.1 MPa for oak and juniper, respectively). These results differed markedly from the forest site study (chapter II, Fig. 4) where oak

exhibited lower water potentials than juniper until mid summer. Water potentials did not differ with canopy level or direction ($P = 0.4200$)

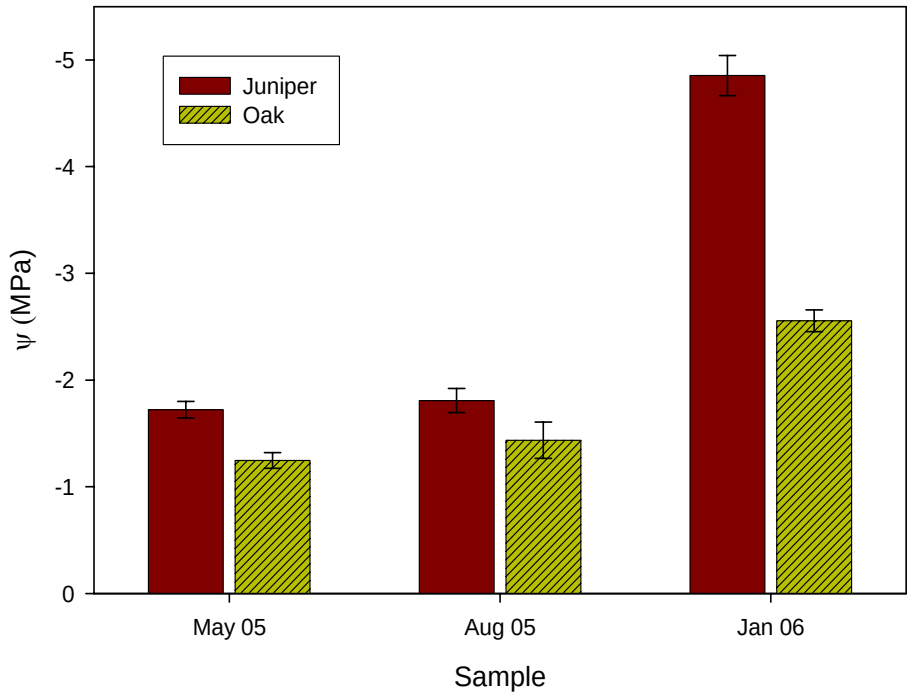

Figure 21. Xylem water potential for Ashe Juniper and live oak in savanna setting. Error bars represent ± SE.

Light Response Curves

Determining light response curves from field data proved problematic. It was not possible to compute curves for many of the samples because of extremely low gas exchange activity. For January 2006, I could not compute light curves for 75% of the samples, in May 2005 19% were not included in the analysis, and in August 2005 3% were not useable. Overall, it was not possible to compute light compensation curves for 32% of my samples. When a leaf exhibited low levels of gas exchange at high light levels, I repeatedly sampled other leaves until it was apparent that no response could be acquired. Care was taken during sampling to check for leaks affecting the IRGAS by ensuring that the sample and reference IRGA stayed matched. The leaf chamber of the Li-6400 was monitored for leaks before initiating a sample. No problems occurred on sampling of oak leaf gas exchange. Because of their cylindrical shape and layered structure, juniper needles were difficult to keep sealed.

Light compensation points were affected by canopy level and direction in May 2005 ($P = 0.0437$, Fig. 22) and August 2005 ($P = 0.0357$, Fig. 23). The northwest ranged (±SE) in May 2005, from 216.50 (±32.87) μmol m^{-2} s^{-1} for the shaded bottom level to 51.9 (±12.74) μmol m^{-2} s^{-1} for the intermediate canopy level. The southeast had the greater light compensation point (183 ±35.87 μmol m^{-2} s^{-1}) for the top level and the minimum (135.28 ±51.13 μmol m^{-2} s^{-1}) for the lowest canopy level. For August 2005 the northwest high was 111.52 (±52.09) μmol m^{-2} s^{-1} for the bottom canopy level, the low was 45.48 (±18.22) μmol m^{-2} s^{-1} for the top layer of the canopy. The southeast ranged from 111.62 (±35.72) at the top level to 29.87 (±5.73) μmol m^{-2} s^{-1} for the bottom level.

Values of light compensation levels were not consistent between month of observation, canopy level, or direction of leaves. There was no significant difference in light compensation point between species at both dates sampled (P = 0.8663, May 2005; P = 0.1194, August 2005).

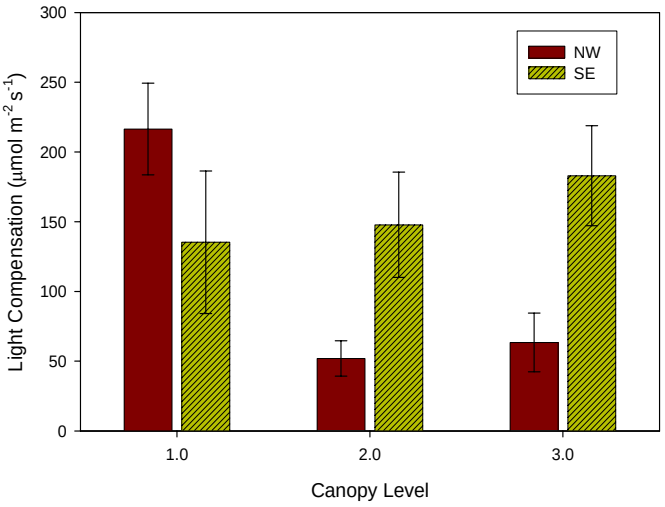

Figure 22. May 2005 light compensation by direction and canopy level for juniper and oak in a savanna setting. Error bars represent ± SE.

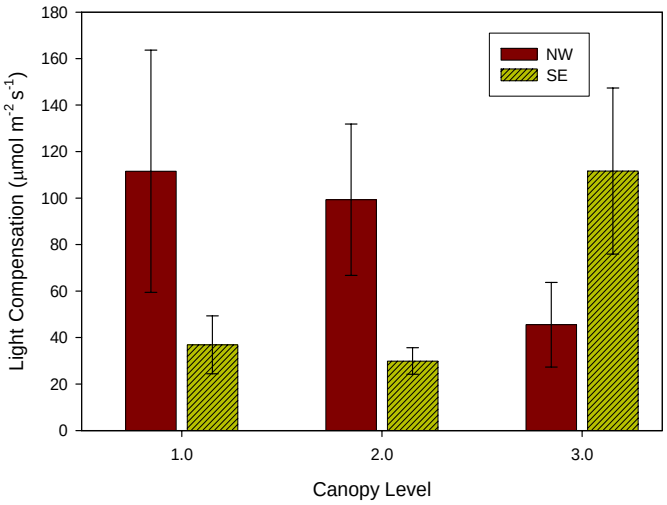

Figure 23. August 2005 light compensation by direction and canopy level for juniper and oak in a savanna setting. Error bars represent ± SE.

For light saturation in August 2005 the relationship between species and direction was significant (Fig. 24, P = 0.0635). The mean saturation points (±SE) for northwest were 553.33 (±131.57) and 451.11 (±66.82) µmol m^{-2} s^{-1} for juniper and oak respectively. The southeast was 269.61 (±45.92) µmol m^{-2} s^{-1} for juniper and 528.89 (±66.82) µmol m^{-2} s^{-1} for oak. Live oak exhibited lower light saturation levels for northwest directed leaves, whereas juniper values were significantly lower for southeast. The analysis of May 2005 data showed light saturation to be affected by canopy level and direction of leaves (Fig. 25, P = 0.0499). Differences mainly occur between the bottom level, and top level in both northwest and southeast direction (Fig. 25). Light

saturation levels for northwest decreased with an increase in canopy height, whereas southeast saturation levels increased with an increase in canopy height. May 2005 northwest light saturation values (±SE) were 606.00 (±71.34), 512.80 (±93.57), and 429.50 (±74.90) µmol m^{-2} s^{-1} for bottom, middle, and top levels, respectively. For the southeast light saturation values were 471.25 (±114.26), 605.60 (±92.89), and 705.60 (±95.47) µmol m^{-2} s^{-1} for the bottom, middle, and top canopy levels, respectively. In January 2006, when the trees had the lowest water potentials measured, I was unable to measure meaningful activity in juniper and therefore not able to compute light response curves. The lower branches were especially non-responsive. For all samples taken in January, where light response curves were calculated, there were no significant differences between light compensation, light saturation, direction, or canopy level (t = 0.728).

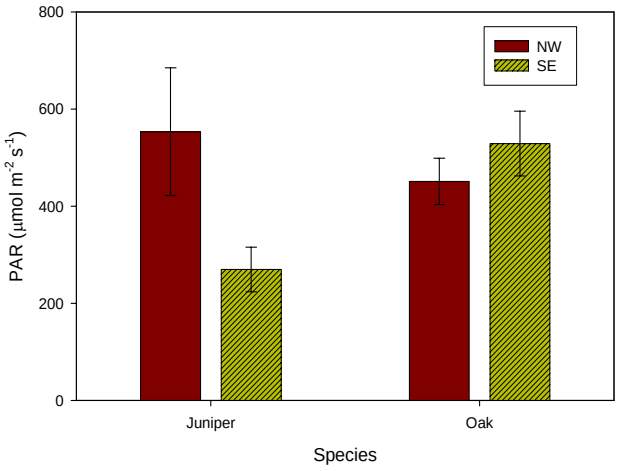

Figure 24. August 2005 light saturation between species and leaf direction. Error bars represent ± SE.

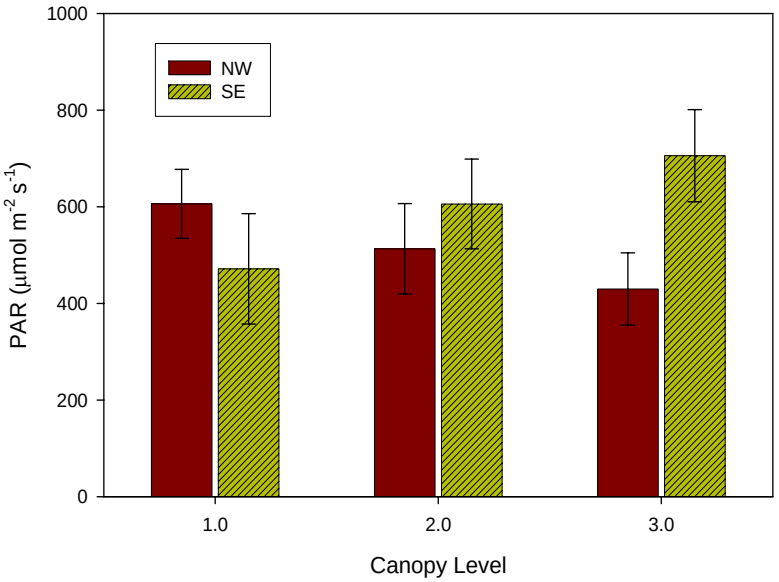

Figure 25. May 2005 light saturation by leaf direction and canopy level. Levels bottom, middle, and top are denoted by 1, 2 , and 3. Error bars represent ± SE.

Discussion

Juniper maintains a smaller leaf area to mass ratio than oak throughout the year (Chapter II). The slight inter-monthly variation shown in juniper specific leaf area (SLA) is most likely only statistically significant rather than biologically. The differences could be explained by small errors in measuring the area of the juniper leaves and also be affected by the variation among leaves. The month of observation and physiological

stage of development affected oak leaves to a greater degree. Live oak leaves are newly expanding in May and have a significantly higher SLA than at other times. The differences in SLA between canopy levels are small, but do indicate that there are at least structural, if not physiological, differences between leaves at the bottom and top of the canopy, representative of shade and sun-lit leaves. Taiz and Zeiger (2002) describe shade leaves as much thinner and therefore having a higher SLA than sun grown leaves. It is possible, that with a larger sample size, differences between the bottom layer and the upper levels would have been even more pronounced than shown in figure 20.

Xylem water potentials for juniper were consistently more negative than for oak in this savanna setting. These results differ somewhat from those of chapter II, where oak had more negative values until mid-summer, but do support the findings of Jackson et al. (1999) and McElrone et al. (2004) that oak more easily maintains xylem water flow. This dissimilarity could be caused by similar size of the trees of the forest site where the oak root structure was less developed and juniper was equally able to take advantage of the high rainfall when the forest site sampling first began. For the savanna site, oak trees were quite large compared to fully grown juniper trees and may have developed the deep penetrating root structure Jackson et al. (1999) measured at 18 m of depth.

At high levels of water stress it became difficult to compute the light response curves. Some curves, mainly in Ashe juniper, were not able to be formed due to inactivity of trees. Responses in changes to light levels were too small and the error of our equipment was larger than actual leaf gas exchange. The Li-6400 manual describes

that when using the Li-6400 to make gas exchange measurements at very low levels of photosynthesis there must be room for an error of about 0.5-1 ppm change gas exchange. In January 2006 the water potentials of both species were lower than they had been all year, with juniper being significantly more negative than oak. Ashe juniper light responses were minimal or non-existing, with the lower branches especially being non-responsive. Live oak showed decreased, but measurable levels of response.

Due to the above obstacles causing a reduction of an already limited sample size, and great variation among individual leaves, our analysis of intra-canopy differences in light responses, are marked by high standard errors and inconsistencies between sample dates. Analysis showed that there were differences in light saturation within the canopies of both juniper and oak depending on canopy level and direction. Owens (1996) showed photosynthetic productivity of the same species to vary with level and direction. Valladares at al. (2000) and Lewis et al. (2000) both looked at light related responses within tree canopies. Lewis et al. (2000) showed in Douglas-fir seedlings that leaf position and orientation within the canopy determined how elevated carbon dioxide affected gas exchange, and that elevated temperature in conjunction with CO_2 and adequate moisture, increased photosynthesis rates at light saturation and light compensation points. Lewis et al. (2000) compared light responses in a canopy of two needle-bearing tree species. Results indicated that light saturated photosynthetic rates, light compensation points, and respiration declined following the canopy gradient from top to the lower branches which is in accordance with expectations of differences between sun and shade leaves as well as direction (Taiz and Zeiger 2002).

The results of this study deviates from most other studies on light responses among leaf positions. For both May 2005 and August 2005 we showed a relationship between light compensation and level and direction of leaves within the canopy. However, during both dates, light compensation increased with a decrease in canopy height for the northwest direction and increased with increasing canopy level. The latter is closer to what we would have expected according to other studies, but even for May 2005, the increase in light compensation was not of significance due to overlapping standard errors. The difference in light compensation for the northwest direction is mainly between the bottom layer of the canopy and the top level, with the shaded leaves having the higher values for light compensation, which would deviate from predictions based on leaf responses from other studies. For August 2005 our data suggest that light saturation for oak trees does not vary based on the cardinal direction a leaf faces in the canopy. On the other hand, saturation points within juniper canopies for August 2005 are markedly higher for the northwest direction than southeast, which is counter-intuitive. Based on these observations I would reject the hypothesis that light saturation would be higher on the southeast side of the canopy, even when the trees are experiencing high water stress. In May 2005, when both tree species were most responsive, light saturation varied significantly, across both species, with leaf direction and level in canopy. Figure 25 shows differences to only be significant between canopy levels 1 and 3 (shade and sun) in both southeast and northwest direction. However, only the southeast directed leaves increase in light saturation as it would be predicted by Lewis et al (2000) study

and Taiz and Zeiger (2002). The decrease in light saturation with increased canopy height for the northwest sided leaves was unexpected.

The results of this study may differ from other studies due to differences between species and site specific environments. Lewis et al. (2000) attributes the observed decline in light compensation point to changes in leaf respiration rather than levels of quantum yield, but a study by Bond et al. (1999) on the same species at a different location indicates the differences to be due quantum yield efficiency. The site specific environment may be of much greater importance to leaf processes than it is often assumed (Lewis et al. 2000).

Oliveira et al. (1996) pointed out that it is a gross assumption to always directly relate individual leaf and branch measurements to whole tree responses, as it may not provide an adequate representation of the heterogeneous population of leaves within a tree canopy. However, when conducting field experiments, methodology is limited by time, people, equipment, and finances. Therefore, general relationships formed from leaf level measurements are usually the most feasible option. With a larger sample size per sample date and with more frequent sampling, it is possible that responses more in line with those of other studies could have been shown. My estimates of light compensation points and light saturation levels, were poor indicators of specific differences between leaves at varying canopy positions. Light is possibly not the single determining factor of canopy and leaf level productivity in this particular environment where water status of the trees also is of great importance.

CHAPTER IV

CONCLUSIONS

It is widely recognized that global climate change and elevated CO_2 will alter the abiotic environment. There is a need to understand ecosystem carbon balance and regulatory mechanisms at all levels of scale (Hanson and Weltzin 2000) so that ecosystem responses to environmental changes can be better predicted. Ecology of invasion is an important area of study. The Edwards plateau represents an ecologically sensitive area, but currently, concerns over water yield take precedence over considerations of possible changes in biodiversity affected by management decisions. This study has through assessment and evaluation of species specific traits attempted to explain which environmental factors and leaf traits may regulate species ability to persist and expand in this area. I found that photosynthesis of shade and sun leaves in a dense juniper/oak forest is significantly different in live oak but not in Ashe juniper. Canopy position in live oak regulates leaf level photosynthesis to a higher degree than in Ashe juniper. Gas exchange of both species decline as water becomes limited, but juniper consistently exhibits lower and less fluctuating rates throughout the year than oak. Ashe juniper does not respond quickly to erratic precipitation events. The consistent low rates of gas exchange and stomatal responses in juniper could indicate shallower rooting structure and/or limitation of hydraulic conductivity, as well as photosynthetic capacity. The higher rates of photosynthesis, transpiration, and stomatal conductivity exhibited by

live oak during drought suggest a deeper rooting pattern than Ashe juniper and access to water stored in the fractured limestone. There were differences in leaf-level water-use efficiency between species, but not to a degree that would explain juniper encroachment on the Plateau.

The forest site study would indicate that leaf gas exchange rates are not very useful in predicting what gives Ashe juniper the invasive potential it appears to have. It seems unlikely that in a semi-arid environment as this, where resources are limited, that only gas exchange characteristics are important and responsible for the invasive success of Ashe juniper. Juniper is more affected by environmental stress, such as limited water, than live oak. Ashe juniper appear to not take up or transpire the suspected amounts of water responsible for reducing the recharge of the Edwards aquifer. Removal of juniper may not be effective in increasing water availability of the area. It may however decrease carbon assimilation. When scaling our results to whole-tree level, the relative contribution of water lost through transpiration and CO_2 taken up, showed that live oak compared to Ashe juniper trees of similar size take up more CO_2 on an annual basis but they also lose about twice the amount of water that juniper loses through transpiration.

The results of the light response analysis are susceptible to errors caused by limited numbers of useful samples acquired. As pertaining to the objective of chapter III it appears, from the results of chapter II, probable that leaf position within live oak canopies affects the photosynthetic capacity and light responses at the leaf level. In juniper the differences in leaf position are less pronounced and leaf level responses depend on environmental factors and whole tree characteristics, such as hydraulic

conductivity, rather than leaf physiological differences affecting gas exchange capacity between leaves of different canopy positions. Xu and Baldocchi (2003) found that photosynthetic capacity, throughout the canopy, usually is very dependent on seasonal and environmental changes, in particular decline in soil water and temperature differences. Our results from this semi-arid environment, where water is limited and frequent stress is caused by temperature, would support that assessment. Both Ashe juniper and live oak lacked a sole dependence on light for productivity and control of leaf level responses. The observations of both chapter II and chapter III show that in an environment where resources are limited, species specific photosynthetic capacity and ability to respond to light decline, as responses within canopies are also related to and dependent upon other environmental controls. In particular, these factors include the ability of the trees to utilize rainfall events, maintain xylem water flow, and access stored water resources. There are no physiological explanations to the encroachment on the Edwards Plateau by particularly Ashe juniper. The main reason to the recent decline in grasslands is grazing and suppression of natural fires.

LITERATURE CITED

Adams, R.P., L.E. Fluornoy, R.L. Singh, H. Johnson, and H. Mayeux. 1998. Invasion of grasslands by *Juniperus ashei*: a new theory based on random amplified polymorphic DNAs, (RAPDs). *Biochemical Systematics and Ecology* 26:371-377.

Aerts, R.. R.G.A. Boot, and P.J.M Van der Aart. 1991. The relationship between above- and belowground biomass allocation patterns and competitive ability. *Oecologia* 87:551-559.

Aerts, R. 1995. The advantage of being evergreen. *Trends in Ecology and Evolution* 10: 402-407.

Auken, O.W. 2000. Shrub invasions of north Americas semiarid grasslands. *Annual Review of Ecology and Systematics* 31:198-215.

Barnes, P.W., S.Y. Liang, K.E. Jessup, L.E. Ruiseco, P.L. Philips, and S.J. Reagan. 2000. Soils, topography and vegetation of the Freeman Ranch. Freeman Ranch Publication Series No. 1, San Marcos, TX: Southwest Texas State University Press.

Blomquist, K.W.. 1990. Selected life history and synecological characteristics of Ashe juniper on the Edwards Plateau of Texas. Master's thesis. Texas A&M University. College Station, TX.

Bond, B.J., Farnsworth, R.A. Coulombe and W.E. Winner. 1999. Foliage physiology and biochemistry in response to light gradients in conifers with varying shade tolerance. *Oecologia* 120:183-192.

Bowman, W. D. and S.W. Roberts. 1985. Seasonal and diurnal water relations adjustments in three evergreen chaparral shrubs. *Ecology* 66:738-742.

Carson, D. 2000. Soil of the Freeman Ranch, Hays County, Texas. Freeman Ranch Publication Series No. 4-2000. San Marcos, TX: Southwest Texas State University Press.

Cohen S., K. Miller, K. Duncan, E. Gregorich, Groffman P., Kovacs P. et al. 2001. North America. *In*: McCarthy J.J., O.F. Canziani, N.A. Leary, D.J. Dokken, and K.S. White [EDS.]. Climate Change: Impacts Adaptation and Vulnerability. Cambridge: Cambridge University Press, p. 745-784.

Cregg, B.M. 1991. Leaf area estimation of mature foliage of Juniperus. *Forest Science* 38:61-67.

Dearing, M.D., A.M. Mangione, W.H. Karasov. 2000. Diet breadth of mammalian herbivores: nutrients versus detoxification constraints. *Oecologia* 123: 397-405.

Deng, X., W.H. Ye, H.L. Feng, Q.H. Yang, H.L. Cao, K.Y. Xu, and Y. Zhang. 2004. Gas Exchange characteristics of the invasive species *Mikania micrantha* and its indigenous congener *M. cordata* (Asteraceae) in South China. *Botanical Bulletin of Academia Sinica* 45:213-220.

Dixon, R. 2000. Climatology of the Freeman Ranch, Hays County, Texas. Freeman Ranch Publication Series No. 3-2000, San Marcos, TX: Southwest Texas State University Press.

Epron, D., E. Dreyer. 1993. Long-term effects of drought on photosynthesis of adult oak trees *Quercus petraea* (Matt.) Liebl. and Q*uercus robur* L. in a natural stand. *New Phytologist* 25:381-389.

Hamerlynck, E.P., and A.K. Knapp. 1994. Leaf level responses to light and temperature in two co-occurring *Quercus* (Fagaceae) species: implications for tree distribution patterns. *Forest Ecology and Management* 68:149-159.

Hanson, P.J. and J.F. Weltzin. 2000. Drought disturbance from climate change: response of United States forests. *The Science of the Total Environment* 262: 205-220.

Herrick, J. D., and R.B. Thomas. 2003. Leaf senescence and late-season net photosynthesis of sun and shade leaves of overstory sweetgum (*Liquidambar styraciflua)* grown in elevated and ambient carbon dioxide concentrations. *Tree Physiology* 23:109-118.

Hicks, R.A., and W.A. Dugas. 1998. Estimating Ashe juniper leaf area from tree and stem characteristics. *Journal of Range Management* 51:633-637.

Jackson, R.B., L.A. Moore, W.A. Hoffmann, W.T. Pockman, and C.R. Linder. 1999. Ecosystem rooting depth determined with caves and DNA. *Proceedings of the National Academy of Sciences* 96:11387-11392.

Jessup, K.E., P.W. Barnes, and T.W. Boutton. 2003. Vegetation dynamics in *Quercus-Juniperus* savanna: an isotopic assessment. *Journal of Vegetation Science* 14: 841-852.

Kattenberg A., F. Giorgi F., H. Grassl, G.A. Meehl, J.F.B. Mitchell, R.J. Stouffer et al. 1996. Climate models-projections of future climate. *In*: Houghton, J.T., L.G.M. Filho, B.A. Callander, N. Harris, A. Kattenberg, and K. Maskell [EDS.]. Climate Change: The Science of Climate Change. Cambridge: Cambridge University Press, p. 287-357.

Kraaij, T., M.D. Cramer. 1999. Do the gas exchange characteristics of alien acacias enable them to successfully invade the fynbos? *South African Journal of Botany* 65:232-238.

Kull O., M. Broadmeadow, B. Kruijt, P. Meir. 1999. Light distribution and foliage structure in an oak canopy. *Trees* 14:55-64.

Lewis, J.D., D. Olszyk, and D.T. Tingley. 1999. Seasonal patterns of photosynthetic light response in Douglas-fir seedlings subjected to elevated atmospheric CO_2 and temperature. *Tree Physiology* 19:243-252.

Lewis, J.D., R.B. McKane, D.T. Tingey, and P.A. Beedlow. 2000. Vertical gradients in photosynthetic light response within and old-growth Douglas-fir and western hemlock canopy. *Tree Physiology* 20:447-456.

McDowell, Susan C.L.. 2002. Photosynthetic characteristics of invasive and noninvasive species of *Rubus* (Rosaceae). *American Journal of Botany* 89:1431-1438.

McElrone, A.D., W.T. Pockman, J. Martinez-Vilalta, and R.B. Jackson. 2004. Variation in xylem structure and function in stems and roots of trees to 20 m depth. *New Phytologist* 163:507-517.

Miller, P.M., L.E. Eddleman, and J.M. Miller. 1995. *Juniperus occidentalis* juvenile Foliage: advantages and disadvantages for a stress-tolerant, invasive conifer. *Canadian Journal of Forest Research* 25:470-479.

Norris, M.D., J.M. Blair, L.C. Johnson, R.B. McKane. 2001. Assessing changes in biomass, productivity, and C and N stores following *Juniperus virginiana* forest expansion into tallgrass prairie. *Canadian Journal of Forest Research* 31:1940-1946.

North, G.R.. 1995. Global climate change. *In*: North, G.R., J.S. Schmandt, and J. Clarkson [EDS]. The Impact of Global Warming on Texas. Austin, TX: University of Texas Press, p. 4-23.

Oliveira, G., O.A. Correia, M.A. Martins-Loução, and F.M. Catarino. 1992. Water Relations of cork-oak (*Quercus suber* L.) under natural conditions. *Vegetatio* 99-100:199-208.

Oliveira, G., C. Werner, and O. Correia. 1996. Are ecophysiological responses influenced by crown position in cork-oak? *Annales des Sciences Forestieres Paris* 53:235-241.

Owens, M. K.; 1996. The role of leaf and canopy-level gas exchange in the replacement of *Quercus virginiana* (Fagaceae) by *Juniperus ashei* (Cupressaceae) in semiarid savannas. *American Journal of Botany* 83:617-623.

Owens, M.K., R.K. Lyons, C.J. Alejandro. 2006. Rainfall partitioning within semiarid juniper communities: Effects of event size and canopy cover. *Hydrological Processes* - In Press.

Owens, M.K. and T.G. Schliesing. 1995. Invasive potential of ashe juniper after mechanical disturbance. *Journal of Range Management* 48:503-507.

Owens, M.K. and M.C. Schreiber. 1992. Seasonal gas-exchange characteristics of two evergreen trees in a semiarid environments. *Photosynthetica* 26:389-398.

Pachepsky, L.B. and B. Acock. 1996. Ad adequate model of photosynthesis – II. Dependence of parameters on environmental factors. *Agricultural Systems* 50: 227-238.

Perkins, S.T. and M.K. Owens. 2003. Growth and biomass allocation of shrub and grass seedlings in response to predicted changes in precipitation seasonality. *Plant Ecology* 168:107-120.

Prioul, J.L. and P. Chartier. 1977. Partitioning of transfer and carboxylation components of intracellular resistance to photosynthetic CO_2 fixation: a critical analysis of the methods used. *Annals of Botany* 41:789-800.

Rayment, M.B., D. Loustau, and P.G. Jarvis. 2000. Measuring and modeling conductances of black spruce at three organizational scales: shoot, branch and canopy. *Tree Physiology* 20:713-723.

Russell, F.L. and N.L. Fowler. 1999. Rarity of oak saplings in savannas and woodlands of the eastern Edwards Plateau, Texas. *Southwestern Naturalist* 44:31-41.

Schwinning, S., O.E. Sala, M.E. Loik, and J.R. Ehleringer. 2004. Thresholds, memory, and seasonality: understanding pulse dynamics in arid/semi-arid ecosystems. *Oecologia* 141:191-193.

Schneider, S.H. 1993. Scenarios of global warming. *In*: Kareiva, P.M., J.G. Kingover, and R.B. Huey [eds]. Biotic Interactions and Global Change. Sunderland: Sinauer Associates, Incorporated, p. 9-23.

Seyfried, M.S., S. Schwinning, M.A. Walvoord, W.T. Pockman, B.D. Newman, R.B. Jackson, and F.M. Phillips. 2005. Ecohydrological control of deep drainage in arid and semiarid regions. *Ecology* 86:277-287.

Smeins, F.E. and L.B. Merrill. 1988. Long term change in a semi-arid grassland. Edwards Plateau vegetation-plant ecological studies in Central Texas. Waco, TX: Baylor University Press.

Taiz, L. and E. Zeiger, 2002. Plant Physiology, Third Edition. Sunderland, Sinauer Associates, Incorporated, p. 175-184.

Takaneaka, A. 1994) A simulation model of tree architecture development based on growth response to local light environment. *Journal of Plant Research* 107: 321-330.

U.S. Soil Conservation Service Staff. 1984. Classification of the soils. *In:* C.D. Batte (ED). Soil survey of Comal and Hays counties, TX. Washington, D.C.: U.S. Department of Agriculture, p. 65-77.

Valladares, F., E. Martinez-Ferri, L. Balaguer, E. Perez-Corona, and E. Manrique. 2000. Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? *New Phytologist* 148:79-91.

Xie, S. and X. Luo,. 2003. Effect of leaf position and age on anatomical structure, photosynthesis, stomatal conductance and transpiration of Asian pear. *Botanical Bulletin of Academia Sinica* 44:297-303.

Xu, L., D. Baldocchi. 2003. Seasonal trends in photosynthetic parameters and stomatal Conductance of blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature. *Tree Physiology* 23:865-877.

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